crement titration method.[8] That both samples of CB-1A possessed the same specificity was demonstrated by complete cross-neutralization of excised uterine strips from appropriately sensitized guinea pigs when tested by the Schultz–Dale method.

When diluted $1:10^6$ (near threshold dilution) both samples of CB-1A gave cutaneous reactions of equal intensity on a castor bean-sensitive subject. A threshold quantity of 0.001 mγ of each sample of CB-1A was required to produce positive passive transfer reactions using serum from a castor bean-sensitive subject.[9]

Experimental[10]

Isolation of CB-1A from Brazilian Castor Bean Pomace.—Brazilian castor bean pomace was obtained from a commercial source in the United States. The sample consisted of broken shells and crushed seeds. The pomace was ground to a coarse powder in a hand grinder in a hood.[11] Experiment showed that the yield of CB-1A obtained directly from the pomace was the same as that obtained after ether extraction.

A preliminary heat treatment of the castor beans, to destroy the ricin toxicity,[12] was carried out as follows: To 3 kg. of ground castor bean pomace was added 6 l. of distilled water. The mixture was heated in an autoclave to 85–92° and maintained at that temperature for one and one-half hours. The suspension was then cooled slightly and an additional 12 l. of water was added. The procedure for isolating CB-1A was then essentially the same as that previously described,[3] except that water extracts from 12 kg. of pomace were combined and worked up together. A further convenient modification was the substitution of pressure filtration through a Seitz sterilizing pad, instead of centrifugation in the Sharples supercentrifuge, to clarify solutions at corresponding points in the procedure.

From a total of 39.9 kg. of pomace, worked up in four lots, 181.3 g. (air dried) of CB-1A was obtained. The four samples of CB-1A were combined, dissolved in 2 l. of water and reprecipitated with five volumes of ethanol at 5°. The recovered CB-1A was dried in a vacuum over calcium chloride. Before analysis the dried CB-1A was ground to pass a 100-mesh sieve and equilibrated with air.

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(8) The dilution of each sample of CB-1A required to produce 2 + contractions in the uterine muscles of sensitized guinea pigs was $1:1.83 \times 10^{11}$.

(9) Details of this method of comparing castor bean allergenic fractions are given in Table III of Paper IX.[3] The serum (W) used for this comparison gave positive passive transfer reactions when diluted $1:10^3$. *Cf.* Coca and Grove, *J. Immunol.*, **10**, 445 (1925). The authors are indebted to Dr. Harry S. Bernton for this serum and to Dorris C. Chambers for the clinical tests.

(10) The authors acknowledge the technical assistance of James H. Shimp in the isolation of CB-1A from the pomace.

(11) Ground pomace is commercially available.

(12) Stillmark[4] and later workers have shown that moist heat destroys the toxicity of ricin. Osborne, Mendel and Harris observed that heating at 60–80° coagulates ricin. Unpublished experiments in this Laboratory have shown that heating water extracts of castor beans destroys their toxic action but an ulcer-producing factor remains. Thus guinea pigs survived subcutaneous injection of 174 M. L. D. of nitrogen of a clarified water extract of castor beans that had been heated for one hour at 77–80°. However, ulcers developed at the site of the injection. Carmicheal (*Soc. Exptl. Biol. Med.*, **24**, 5 (1926)) previously reported that ricin solutions were detoxified by sodium ricinoleate but that ulcers always formed at the site of the injection. In protocols of later work by Carmicheal (*J. Pharmacol.*, **35**, 193 (1929); *ibid.*, **35**, 223 (1929)) it is apparent that ulcers sometimes formed on injection of ricin solutions detoxified by other means. Further work is needed to clarify the nature of this ulceration factor produced by detoxifying ricin solutions or castor bean extracts.

The Sulfonation of Acetophenone

BY E. H. WOODRUFF

It was reported[1] that the alkali fusion of acetophenone disulfonyl ch. ride gave *m*-hydroxybenzoic acid, confirming the statement of Suter and Weston[2] and that only one sulfonic acid group had entered the ring. Weston and Suter,[3] in a more detailed study, isolated only salicylic acid from the fusion of their acid chloride, showing it to be acetophenone 2,α-disulfonyl chloride.

To clarify this contradiction a further examination of the experimental data (not previously reported) yields the following information:

When added to cooled chlorosulfonic acid and then heated at 110° acetophenone yields an ether-insoluble compound, m. p. 195–196° (from carbon tetrachloride), identical with that previously reported.[3,4] This material on fusion gives salicylic acid. If, however, acetophenone is added to chlorosulfonic acid already heated to 110°, upon pouring onto ice almost no insoluble precipitate is obtained. Upon working up the aqueous solution a disodium disulfonate is obtained which on fusion with alkali gives *m*-hydroxybenzoic acid. Thus the *m*-hydroxybenzoic acid does not result from the fusion of the disulfonyl chloride, as the previous report from this Laboratory would indicate, but from another water-soluble product of the sulfonation. The aqueous solution from which the disulfonyl chloride was obtained has not been worked up in a similar manner. It would appear, however, that acetophenone may sulfonate in either the ortho or meta position when treated with chlorosulfonic acid. A low temperature during the mixing of the reactants favors the formation of the ortho derivative. Whether the meta isomer is formed during the process giving the best yield of the ortho isomer has not been demonstrated but in view of the low yield of the ortho isomer it is not unlikely that such is the case. The behavior of acetophenone toward sulfonation appears to duplicate that of nitration[5] where both the ortho and meta nitroacetophenones are formed.

Experimental

Acetophenone 2-α-Disulfonyl Chloride.—This was prepared by the addition of acetophenone to chlorosulfonic acid kept at room temperature and then heated to 110° for one hour[4]; yield 21.5%, m. p. 195–196° uncor., crystallized from carbon tetrachloride.

Acetophenone 3-α-Disulfonic Acid Disodium Salt.—To 1 kg. (8.60 moles) of chlorosulfonic acid heated to 110° with stirring, 168 g. (1.4 moles) of acetophenone was added over a period of one hour. The temperature rose to 120° during the addition and was kept there an additional hour. After cooling to 10°, the material was added to 4 kg. of cracked ice. One hundred cc. of chloroform was added and the solution filtered with suction. Upon heating the solution to 80° with a current of steam, 1880 g. (5.8 moles)

(1) Woodruff, THIS JOURNAL, **64**, 2859 (1942).

(2) Suter and Weston, *ibid.*, **61**, 233 (1939).

(3) Weston and Suter, *ibid.*, **61**, 389 (1939).

(4) Riesz and Frankfurter, *Monatsh.*, **50**, 68 (1928).

(5) Reese, *Chem. Rev.*, **14**, 90 (1934).

of hydrated barium hydroxide was added. The barium sulfate was removed and 250 g. of sodium carbonate added. Upon evaporation to dryness, 375 g. of crude sodium salt was obtained.

m-**Hydroxybenzoic Acid.**—One hundred twenty-nine and six-tenths grams (0.4 mole if pure) of the above salt was fused with a mixture of 200 g. of sodium hydroxide and 168 g. of potassium hydroxide at 310° for two hours. After cooling the solid was dissolved in 800 cc. of water and 700 cc. of concentrated hydrochloric acid added. On cooling the aqueous solution was extracted with three 200-cc. portions of ether. The ether on evaporation gave 20 g. (36%) of crude acid m. p. 172–182°; recrystallized twice from benzene–ether, m. p. 202° uncor.

When treated with methyl sulfate and alkali *m*-methoxybenzoic acid was obtained, m. p. 101–102°; neutral equivalent calcd., 152.0; found, 150.0.

THE UPJOHN COMPANY
KALAMAZOO, MICHIGAN

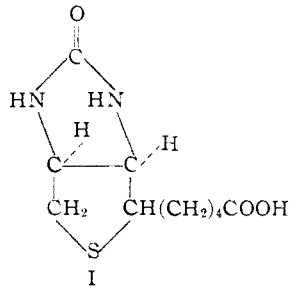
RECEIVED JUNE 8, 1944

COMMUNICATIONS TO THE EDITOR

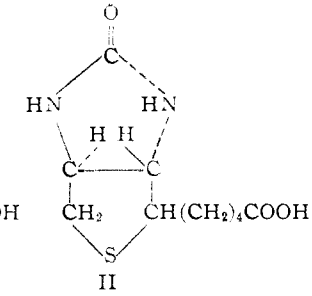
BIOTIN. III. *cis* and *trans* FORMS RELATED TO *dl*-BIOTIN

Sir:

It was stated in a previous publication[1] that it was believed that compounds having two five-membered saturated heterocyclic nuclei fused through adjacent carbon atoms, as in biotin, would exist only in *cis* forms of the rings, as in structure I.



I

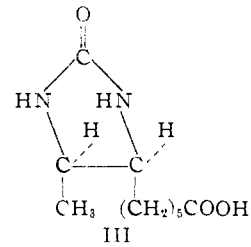


II

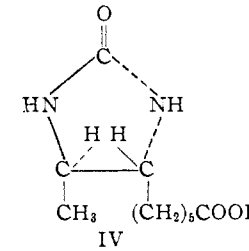
Originally two racemates related to biotin, namely, *dl*-biotin, m. p. 232° (*Anal.* Calcd. for $C_{10}H_{16}N_2O_3S$: C, 49.16; H, 6.60; N, 11.46. Found: C, 49.33; H, 6.39; N, 11.68), and *dl*-allobiotin, m. p. 194–196° (*Anal.* Calcd. for $C_{10}H_{16}N_2O_3S$: C, 49.16; H, 6.60; N, 11.46. Found: C, 49.36; H, 6.50; N, 11.39), were obtained, one of which yielded biotin on resolution. The series of reactions[2] which led to the formation of these racemates is described in another communication. A third racemate, *dl-epi*allobiotin (decomposes without melting starting at 195°) (*Anal.* Calcd. for $C_{10}H_{16}N_2O_3S$: C, 49.16; H, 6.60; N, 11.46. Found: C, 49.23; H, 6.75; N, 11.21), having the structure of biotin has been derived from the reduction product of the dehydro isomer[2] melting at 162–163° The other reduction product from this isomer yielded *dl*-allobiotin.

Thus, three racemates corresponding to six of the eight theoretically possible isomers are known. It is evident that one or two of the known racemic pairs must have a *trans* configuration of its nitrogen atoms as represented by structure II.

This new racemate has been correlated to *dl*-allobiotin[2] by hydrogenolysis[3] with Raney nickel catalyst. Both the new racemate and *dl*-allobiotin gave the same desthio derivative, III or IV, which is called *dl*-desthioallobiotin; m. p. 165–166° (*Anal.* Calcd. for $C_{10}H_{18}N_2O_3$: C, 56.05; H, 8.47; N, 13.08. Found: C, 55.86; H, 8.25; N, 12.76.



III



IV

dl-Biotin gave *dl*-desthiobiotin which also melted at 165–166° (*Anal.* Calcd. for $C_{10}H_{18}N_2O_3$: C, 56.05; H, 8.47; N, 13.08. Found: C, 56.04; H, 8.52; N, 13.22). However, these two compounds showed a mixed melting point depression of twenty degrees. Dr. Jacob L. Stokes of this Laboratory found that *dl*-desthioallobiotin was inactive for the growth of yeast, while *dl*-desthiobiotin was one-half as active as *d*-desthiobiotin.[4]

From these results it is evident that the new racemate is epimeric at carbon atom 2 of *dl*-allobiotin; therefore, it will be called *dl-epi*allobiotin.

The fact that *d*-desthiobiotin methyl ester[3] and *d*-desthiobiotin ($[\alpha]^{31}D$ +10.4 (*c*, 1.7525 in 0.1 *N* sodium hydroxide)) have a low but definite optical activity is evidence that inversion of the nitrogen atoms did not take place during the hydrogenolysis. Furthermore, the latter compound does not agree in melting point[4] or mixed melting point with either of the *dl* derivatives.

(1) Paper I, Harris, Wolf, Mozingo and Folkers, *Science*, **97**, 447 (1943).

(2) Harris, *et al.*, Paper II, THIS JOURNAL, **66**, 1756 (1944).

(3) du Vigneaud, *et al.*, *J. Biol. Chem.*, **146**, 475 (1942); Mozingo, *et al.*, THIS JOURNAL, **65**, 1013 (1943).

(4) Melville, Dittmer, Brown and du Vigneaud, *Science*, **98**, 497 (1943).